

Protective effects of coenzyme Q10 nanoparticles on dichlorvos-induced hepatotoxicity and mitochondrial/lysosomal injury

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Abstract

Development of biocompatible antioxidant nanoparticles for xenobiotic-induced liver disease treatment by oral or parenteral administration is of great interest in medicine. In the current study, we demonstrate the protective effects of coenzyme Q10 nanoparticles (CoQ10-NPs) on hepatotoxicity induced by dichlorvos (DDVP) as an organophosphate. Although CoQ10 is an efficient antioxidant, its poor bioavailability has limited the applications of this useful agent. First, CoQ10-NPs were prepared then characterized using dynamic light scattering (DLS) and transmission electron microscopy (TEM). In DDVP-treated and non-treated hepatocytes in the presence of CoQ10-NPs, cell viability, the level of reactive oxygen species (ROS), lipid peroxidation (LPO), mitochondrial membrane potential (MMP), lysosome membrane integrity, and cellular glutathione (GSH) content were measured. The prepared CoQ10-NPs were mono-dispersed and had narrow size distribution with average diameter of 54 nm. In the in vivo study, we evaluated the enzymes, which are involved in the antioxidant system for maintenance of normal liver function. In comparison to nonparticulate CoQ10, the CoQ10-NPs efficiently decreased the ROS formation, lipid peroxidation and cell death. Also, particulate form of CoQ10 improved MMP, GSH level and lysosome membrane integrity. In the in vivo study, we revealed that CoQ10-NPs were better hepatoprotective than its nonparticulate form ($P < .05$). Altogether, we propose that the CoQ10-NPs have potential capability to be used as a therapeutic and prophylactic agent for poisoning that is induced by organophosphate agents, especially in the case of DDVP. Furthermore, these positive remarks make this nanoparticle amenable for the treatment of xenobiotic-induced liver diseases.

KEYWORDS

Nanoparticles, Dichlorvos, Coenzyme Q10, Organophosphates, Oxidative Stress, Cytotoxicity

1 | INTRODUCTION

The exposure to organophosphates such as dichlorvos (DDVP) results in prominent neurotoxicity and hepatotoxicity.^{1,2} Liver, with crucial importance in metabolism and detoxification of chemicals, seems to be a potential site for organophosphates especially DDVP-induced damages;^{3,4} however, it has been recognized that chronic and sub-chronic hepatotoxicity of organophosphates is closely linked to the oxidative stress.^{5–7}

DDVP is an organophosphate pesticide that effectively acts against various types of agricultural, household, public health and fish

farming pests and works as plasticizers and flame retardants in industry.⁸ Despite the regulations and/or limitations by the United States Environmental Protection Agency (EPA), World Health Organization (WHO) and Agency for Research on Cancer on human health risk,⁹ it is still used over the world, particularly in developing countries, because of its broad-spectrum actions. Previous studies have demonstrated that in the mitochondria isolated from rat brain exposed to DDVP, GSH level decreases and content of ROS significantly increases.^{1,10}

It has been recognized that powerful antioxidants such as Coenzyme Q10 (CoQ10), which can directly scavenge ROS and indirectly regenerate cellular vitamin E and C, are able to reduce the lipid

peroxidation (LPO) in cellular membranes.¹¹ CoQ10, also known as ubiquinone, is a salient endogenous lipid-soluble and vitamin-like substance that acts as a proton-electron carrier in the mitochondrial electron transport chain and consequently supply energy need (ATP) of cell.^{12,13} Furthermore, it has been presented that CoQ10 had hepatoprotective impacts against xenobiotic-associated cellular damage and oxidative stress in several cases such as statins, acid salicylic acid, thioacetamide, tetrachloride carbon and doxorubicin.^{14–16}

In spite of having protective role against toxicities induced by xenobiotics, CoQ10 represents significant lipid solubility and low bioavailability that limit its clinical applications; therefore, higher doses are needed to be administered orally for clinical applications.¹⁷ It is worth noting that researches have achieved obvious therapeutic effectiveness in humans only in the long term and high dose CoQ10 administration, which forces researchers to seek strategies for overcoming these limitations.¹⁸

In this study, we aimed to test and compare the hepatoprotective and prophylactic effects of the nonparticulate CoQ10 on DDVP-induced hepatotoxicity and oxidative stress in isolated rat liver cells. In these cells, viability, ROS formation, lipid peroxidation, GSH level, and mitochondrial/lysosomal function were studied and for complementary investigations to confirm *in vitro* results we carried out *in vivo* assays that could value the DDVP-induced liver damage and effects of CoQ10 and CoQ10-NPs.

2 | METHODS AND MATERIALS

2.1 | Chemicals

Thiobarbituric acid (TBA), trypan blue, GSH, and phenobarbital were prepared from Merck Chemical Company (Darmstadt, Germany). 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES) were prepared from Acros (New Jersey, USA). Other agents were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO).

2.2 | Synthesis of CoQ10-NPs

CoQ10 nanoparticles were produced by precipitation method.¹⁸ The solvent for 10 mg of CoQ10 was ethanol (3.0 mL) which is the organic phase of the solution. The obtained solution was poured into 27.0 mL of double distilled water and stirred at 14,000 rpm for 15 s (Heidolph, silence crusher, Germany). After evaporation of ethanol by rotary vacuum at 40°C water bath, a freeze dryer was used to lyophilize of remaining fraction. For the preparation of the coarse suspension, 30.0 mg of coenzyme Q10 was added in 30.0 mL of cold distilled water (containing 10% v/v ethanol). The coenzyme Q10 content of both was adjusted to about 1 mg/mL.

2.3 | Characterization of the prepared CoQ10-NPs

The particle size distribution and zeta potential of the CoQ10-NPs were determined by dynamic light scattering (DLS) using a nano/zetasizer (Malvern Instruments, Worcestershire, UK, model Nano ZS) at room temperature. The synthesized nanoparticles were characterized

for their size and structure by transmission electron microscopy (Zeiss, Germany). The samples stained with 0.5% (w/v) phosphotungstic acid on copper grids for observation before TEM analysis.

2.4 | Animal treatment

Adult Male Wistar rats (body weight 280–320 g) were kept in standard laboratory conditions with a 12-h light/dark cycle by the animal research center of Tabriz University of Medical Science, Tabriz, Iran. The animals received humane care according to the ethical guidelines of the National Institute of Health (NIH publication No. 85-23, revised 1985) and guidelines of Tabriz University of Medical Sciences, approved by a Committee of Animal Experimentation (No: 91/2-2/5/4 Dec 2012).

2.5 | Isolation and incubation of rat hepatocytes

Rat hepatocytes were isolated with collagenase-perfusion method following the exclusion of calcium ions (Ca^{2+}) with a chelator (EGTA 0.5 mM) as described previously.¹⁹ The isolated hepatocytes in a density of $10^6/\text{mL}$ were then suspended in the Krebs-Henseleit buffer ($\text{pH} = 7.4$) in continuously rotating round bottom flasks supplemented with 12.5 mM HEPES, in a 37°C water bath under an atmosphere of carbogen gas (95% O_2 and 5% CO_2).^{20,21} Hepatocytes were preincubated for 30 min prior to addition of chemicals to adopt the medium. Freshly prepared chemical stock solutions ($100 \times$ concentrated for the water solutions or $1000 \times$ concentrated for the methanolic solutions) were utilized in this experiment. To avoid either nontoxic or very toxic circumstances, we used LC_{50} concentrations (1 mM) for DDVP.

2.6 | Cell viability

Hepatocyte viability was evaluated microscopically by trypan blue (0.1%, w/v) exclusion test [17]. Sampling was performed every 60 min during a 3 h of incubation period while 85–90% of hepatocytes were viable before use

2.7 | Reactive oxygen species (ROS) formation assay

To assess the amount of hepatocyte ROS generation after DDVP was added to 2,7-dichlorofluorescein diacetate (DCFH-DA) containing hepatocytes to hepatocytes as it infiltrates hepatocytes and is hydrolyzed to non-fluorescent dichlorofluorescein (DCF) by an intracellular esterase. DCFH then reacts with reactive species and changes into highly fluorescent dichlorofluorescein. In 1, 2 and 3 h time points over isolation, 1 mL (10^6 cell) of hepatocytes suspension was picked up and centrifuged at 3000g for 1 min. Fluorescence intensity per 10^6 cells as a result of ROS production was measured using a Jasco R_FP-750 spectrofluorometric (Jasco Corporation, Tokyo, Japan) with excitation and emission wavelengths of 500 and 520 nm, respectively.²²

2.8 | Determination of lipid peroxidation

Lipid peroxidation (LPO) of the hepatocytes was determined through measuring thiobarbituric acid reactive substances (TBARS).²³ Briefly,

1 mL of hepatocytes suspension (10^6 cells/mL) was treated with 250 μ L trichloroacetic acid (TCA 70% w/v) and centrifuged at 3000g for 15min; then 1mL of thiobarbituric acid (TBA 0.8% w/v) was added to supernatant while boiling for 20 min. The absorbance was determined at 532 nm in an Ultrospec® 2000 UV spectrophotometer.^{24,25}

2.9 | Mitochondrial membrane potential (MMP) assays

The cationic dye, rhodamine 123 accumulates in intact mitochondria. When MMP is altered by any xenobiotic, the facilitated diffusion of dye is diminished leading to the elevation of the amount of rhodamine 123 in media. At the given times, aliquots of 1 mL samples of the cell suspension were taken and centrifuged at 1000 g for 1 min. Then, the cell pellet was resuspended in 2 mL of fresh incubation medium containing 1.5 μ M of rhodamine 123 and incubated at 37°C in a water bath with a gentle shaking. Hepatocytes were separated by centrifugation at 3000 g for 1 min and the amount of rhodamine 123 remaining in the incubation medium was calculated using a Jasco FP-750 fluorescence spectrophotometer set at 490 nm excitation and 520 nm emission wavelengths.^{26,27}

2.10 | Lysosomal membrane integrity assay

Acridine orange redistribution was utilized to assess the hepatocyte lysosomal membrane stability.²⁸ In brief, 0.5 mL of the prestained cell suspension with acridine orange (5 μ M) was picked up from the incubation medium and was centrifuged 1 min at 800 g. Afterwards, the cell pellet was resuspended in 2 mL of fresh incubation buffer. In order to remove the fluorescent dye from the media, a two time washing process was performed. The distribution of dye in the cell suspension was then assessed fluorimetrically using a Jasco FP-750 fluorescence spectrophotometer set at 495 nm excitation and 530 nm emission wavelengths.

2.11 | Measurement of intracellular GSH and GSSG

Reduced glutathione (GSH) and glutathione disulfide (GSSG) in isolated hepatocytes was measured in deproteinized samples (5% metaphosphoric acid) after derivatization with iodoacetic acid and 1-fluoro-2,4-dinitrobenzene,²⁹ using a μ Bondapak NH2 column, by HPLC (Water Associates, Milford, MA).

To do the experiment, the reaction of S-carboxymethyl derivatives of free thiols with iodoacetic acid was followed by conversion of free amino groups to 2, 4-dinitrophenyl derivatives through reaction with FDNB. Briefly, 0.8 mL of the cell suspension was spun at 50 g for 40 s, and the cell pellet was resuspended in 0.8 mL of fresh Krebs-Hensleit medium. Deproteinization of the cell suspension was performed with the addition of 0.2 mL volume of 25% metaphosphoric acid with a further centrifugation at 100g for 5 min. In the presence of excess sodium bicarbonate, the mixture of 0.5 mL of supernatant and 0.05 mL of iodoacetic acid was sealed and left in the dark and room temperature for 1 h. Eventually, 0.5 mL of FDNB solution (1.5%, v/v in ethanol) was added to the sample, left in the dark for 24 h at room temperature and then was examined by HPLC.

2.12 | In vivo studies

The animals were divided into following five groups (5 animals in each group) for 30 days: Group 1 (control): vehicle (0.9% saline solution) via oral gavage. Group 2: DDVP-treated animals (1.6 mg/kg body weight) (1/50 LD oral dose) per day. Group 3: NAC (100 mg/kg body weight per day)³⁰ + DDVP (1.6 mg/kg body weight per day) via oral gavage. Group 4: CoQ10 (50 mg/kg body weight per day)³¹ + DDVP (1.6 mg/kg body weight per day) via oral gavage. Group 5: CoQ10-NPs (50 mg/kg body weight per day) + DDVP (1.6 mg/kg body weight per day) via oral gavage. Antioxidants intake was done 3 h prior to DDVP-administration. After the completion of treatment, all animals were fasted for 12 h, then blood was sampled via the abdominal vena cava using pentobarbital anesthesia. Serum alanine transaminase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGT) activities were measured with a commercial kit. To assess the histopathological changes, paraffin-embedded liver sections were stained with heamatoxyline and eosine (H&E) before light microscopy.

The livers were removed, rinsed in ice-cold physiological saline (0.9% NaCl) and homogenized using a Teflon homogenizer (Heidolph Silent Crusher M). The liver homogenate was centrifuged at 1600 g for 15 min at 4°C to prepare supernatants. Malondialdehyde (MDA) concentration in tissues was considered as nmol MDA per milligram of protein of liver tissue. Catalase (CAT) and superoxide dismutase (SOD) activities were measured according to the methods described by Aebi and Beauchamp respectively.^{32,33} Total glutathione peroxidase (GPx) was measured as unit/mg protein according to the method of Flohe et al.³⁴ The protein concentration was assed by the method of Lowry et al.³⁵

2.13 | Statistical analysis

Data are presented as the Mean \pm SD for at three independent experiments. Results were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to assess significance. Results with values for $P < .05$ were taken as statistically significant.

3 | RESULTS

3.1 | Particle size analysis and morphological characterization

As shown in DLS plot, it is evident that prepared NPs have a narrow size distribution and are highly monodispersed (Figure 1A). Moreover, the CoQ10-NPs displayed an effective diameter of 54 nm, while zeta potential was found to be -24 mV (Figure 1A). Additionally, TEM micrograph shows that CoQ10-NPs are morphologically spherical and uniform in size distributions, which was confirmed the results of DLS analysis (Figure 1B). As presented in Figure 2, the appearances of coarse suspensions and the CoQ10-NPs were different. It was found that all nanocrystals were homodispersed; on the contrary, the bulk CoQ10 dispersed in suspension was quite unstable after 2 days, while the 54 nm-sized nanocrystals were physically stable for during 3 month at room temperature.

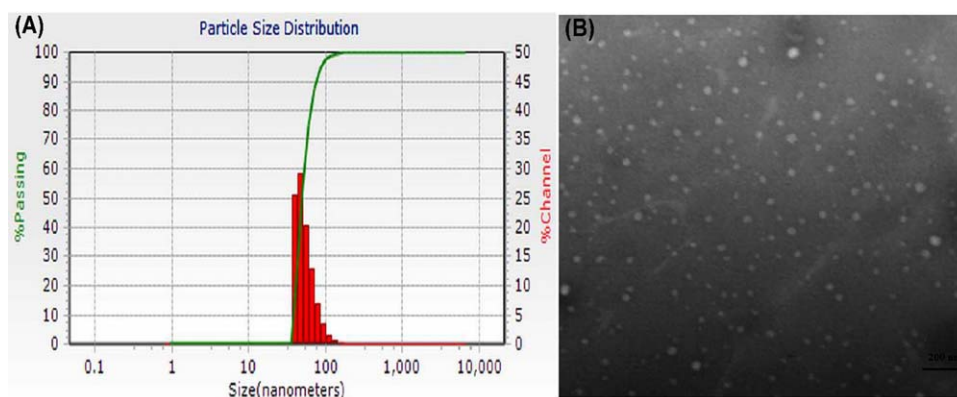


FIGURE 1 Dynamic light scattering (DLS) of the CoQ10-NPs (A). TEM image of Coenzyme Q10 nanoparticles. The average size of CoQ10-NPs was 54 (B) [Color figure can be viewed at wileyonlinelibrary.com]

3.2 | Cytotoxicity caused by DDVP

1mM of DDVP showed 50% cell death (LC50) in a dose depended manner as determined by the trypan blue exclusion test in 120 min of incubation (data not shown). 1mM as LC50 was applied to investigate mechanisms of cytotoxicity and alterations in biochemical markers. In comparison with control hepatocytes, 1mM of DDVP increased hepatocyte membrane lysis significantly (Figure 3). 300 μ M of CoQ10 and 200 μ M of NAC effectively reduced DDVP-induced cell death (Figure 3). The same concentration (300 μ M) of CoQ10-NPs and CoQ10 was used in order to compare the protective effects of these agents. As presented in Figure 4, DDVP-induced toxicity in CoQ10-NPs treated hepatocytes were significantly reduced in comparison to the CoQ10 treated hepatocytes.

3.3 | ROS formation and lipid peroxidation

As shown in Figure 4, incubation of hepatocytes with DDVP caused significant increase in dichlorofluorescein (DCF) fluorescence in

comparison to normal cells. Also, DDVP toxicity was associated with the peroxidation of membrane lipids (Figure 5). Treatment with antioxidants (NAC and CoQ10) protected hepatocytes against DDVP-induced ROS formation and LPO (Figures 4 and 5). Moreover, CoQ10 nanoparticles substantially decreased the ROS and LPO levels in comparison with free CoQ10 ($P < .05$) (Figures 5 and 6).

3.4 | The effects of DDVP on mitochondrial membrane potential

Measuring of fluorescent probe rhodamine 123 as main indicator of mitochondrial membrane potential examines the role of ROS formation in mitochondrial damage. Administration of CoQ10 and NAC significantly decreased the DDVP (1mM) induced mitochondrial membrane potential collapse (Figure 6). Also, nanoparticles of CoQ10 significantly reduced MMP alteration in comparison to nonparticulate form of CoQ10 ($P < .05$) (Figure 6).

3.5 | DDVP-induced lysosomal membrane damage

Incubation of acridine orange (a lysosomotropic agent) loaded hepatocyte with DDVP, resulted in a significant release of the fluorescent dye into the cytosolic fraction within 1 h representing a lysosomal membrane damage (Figure 7). DDVP-induced acridine orange redistribution was again prevented by NAC, CoQ10 and CoQ10-NPs (Figure 7). CoQ10-NPs treated group exhibited a significant reduction in the lysosomal leakiness in comparison with CoQ10 treated group (Figure 7). All of these compounds did not show any marked effects effect ($P < .05$) on acridine orange redistribution from lysosomes to cytosol in normal cells.

3.6 | The effects of DDVP on GSH/GSSG levels

Incubation of hepatocytes with DDVP (1mM) potentiate glutathione depletion of GSH which was significantly prevented by CoQ10 and NAC ($P < .05$) (Table 1). Interestingly, administration of CoQ10-NPs reduces the glutathione reduction profoundly more than free CoQ10 in isolated hepatocytes (Table 1). All these findings indicate the occurrence of oxidative stress after DDVP administration in isolated rat.



FIGURE 2 Suspensions of bulk drugs and coenzyme Q10 nanocrystals after storage for 2 days at room temperature. Note: From left to right: CoQ10 nanocrystals and free CoQ10 respectively. The CoQ10 content of two formulations was about 1 mg/mL. [Color figure can be viewed at wileyonlinelibrary.com]

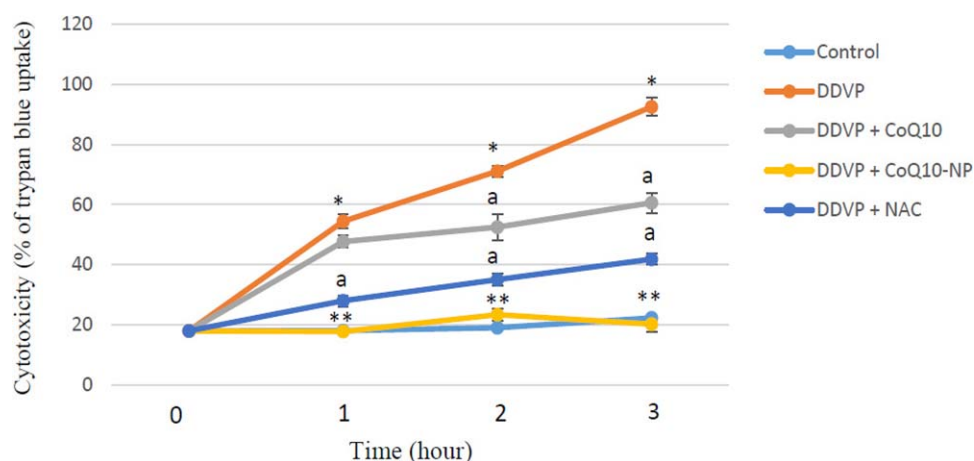


FIGURE 3 DDVP-induced cell cytotoxicity (1mM) and the effect of antioxidants: CoQ10 (300 μ M), NAC (200 μ M) and CoQ10-NPs (300 μ M) in isolated rat hepatocytes. *Significantly higher than control hepatocytes ($P < .05$). ^a CoQ10 (1 mM) significantly decreased hepatocyte membrane lysis compared to DDVP treated hepatocytes. **Co10-NPs (1 mM) significantly decreased hepatocyte membrane lysis compared to DDVP- and CoQ10-treated hepatocytes. Data are shown as mean \pm SD for at least three different experiments. [Color figure can be viewed at wileyonlinelibrary.com]

3.7 | In vivo results

ALT, AST and GGT as main markers of liver function in different treated groups are presented in Table 2. DDVP administration increased the levels of AST, ALT, and GGT to higher extend when compare to the control group ($P < .05$). In contrast pretreatment of rats with NAC, CoQ10 and CoQ10-NPs significantly reduced the above-mentioned alterations ($P < .05$). Moreover, AST, ALT and GGT levels were significantly lower in of CoQ10-NPs ($P < .05$) (Table 2). The histopathological results showed that DDVP administration causes hepatocyte degeneration, fatty changes and inflammatory cell infiltration which was significantly improved after CoQ10 and also CoQ10-NPs. However, this effect was in a greater extent in CoQ10-NP treated group (Figure 8).

As shown in Table 3, DDVP-intoxication significantly plummeted the activity of the SOD, CAT, and GPx enzymes compared with the normal control ($P < .05$). While, pretreatment of rats with NAC, CoQ10 and CoQ10-NPs significantly improved liver antioxidant content ($P < .05$). Also, the result of this study showed that particulated CoQ10 had a more beneficial effect in antioxidant activity of DDVP-intoxicated livers.

4 | DISCUSSION

Nano antioxidants like CoQ10 nanoparticles are safe, biocompatible, and biodegradable with no toxicity and possessing intrinsic antioxidant properties in attenuating of free radical induced oxidative damage.

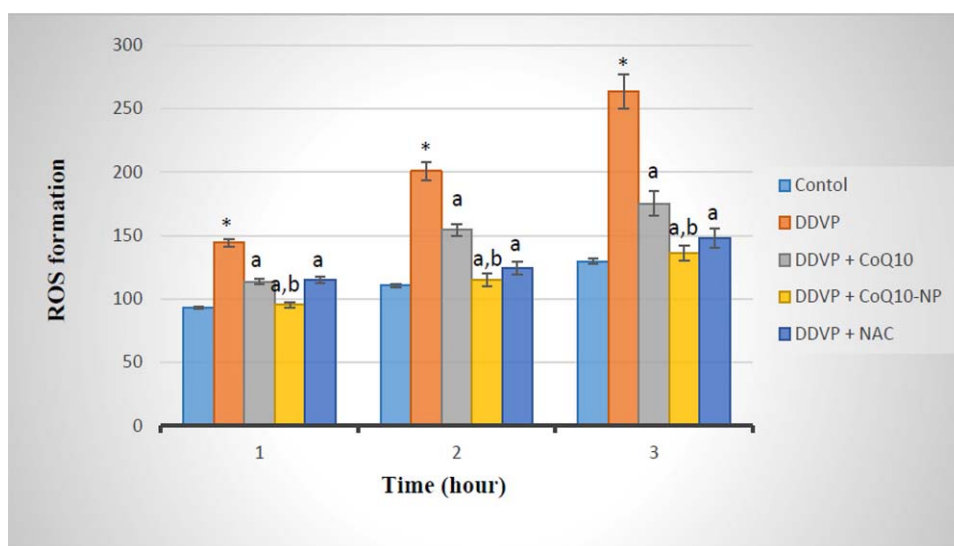


FIGURE 4 Reactive oxygen species formation induced by DDVP and the protective effects of CoQ10, CoQ10-NPS and NAC. Isolated rat hepatocytes (10⁶ cells/mL) were incubated at 37°C in rotating round bottom flasks with 95% O₂ and 5% CO₂ in Krebs-Henseleit buffer (pH 7.4). *Different from control group ($P < .05$). ^aSignificantly different from DDVP-treated hepatocytes ($P < .05$). ^{a,b}Significantly different from DDVP and CoQ10 treated cells ($P < .05$). Data are shown as mean \pm SD for at least three different experiments. [Color figure can be viewed at wileyonlinelibrary.com]

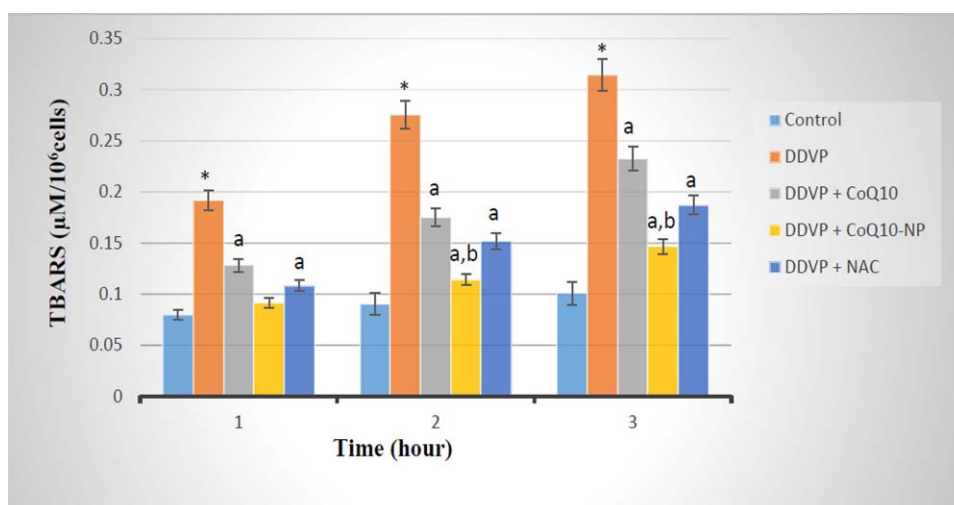


FIGURE 5 TBARS formation induced by DDVP and the protective effects of CoQ10, CoQ10-NPS and NAC. Isolated rat hepatocytes (10^6 cells/mL) were incubated at 37°C in rotating round bottom flasks with 95% O_2 and 5% CO_2 in Krebs–Henseleit buffer (pH 7.4). *Different from control group ($P < 0.05$). ^aSignificantly different from DDVP-treated hepatocytes ($P < 0.05$). ^{a,b}Significantly different from DDVP and CoQ10 treated cells ($P < 0.05$). Data are shown as mean \pm SD for at least three different experiments. [Color figure can be viewed at wileyonlinelibrary.com]

Among several chemical techniques for the synthesis of nanoparticles, precipitation method is a simple and quick preparative method and also size-controllable approach. The precipitation process offers advantages like simplicity, low cost, high purity of product and not requiring organic solvents³⁶. In this study, we used the nanoprecipitation technique that possesses numerous benefits, which offers reproducible particle size with a narrow distribution (PDI value of 0.18) and is comparatively straightforward and rapid.

Preparing reproducible particle size and being a rapid technique makes nanoprecipitation a favorite method to produce nanomaterials and we also used in this experiment.^{37–39} The smaller nanoparticles are

made, the larger surface area is provided leading to enhanced efficacy and increased agent bioavailability. Besides, it appears that original physicochemical properties are boosted with nanoparticulation, providing the crystalline structure which might alter the release profile of nonparticulate CoQ10.

The intensity-weighted mean diameter of the bulk population is measured with DLS method or photon correlation spectroscopy (PCS). As presented in Figure 1A, the diameter for CoQ10-NPs was calculated to be 54 and the prepared CoQ10-NPs had a narrow size distribution with a spherical and uniform morphology (Figure 1B).

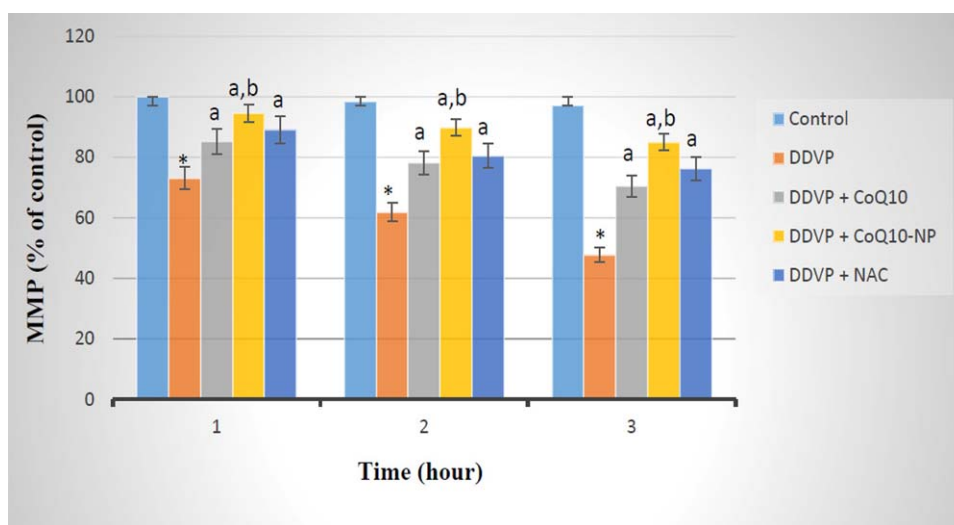


FIGURE 6 Mitochondrial depolarization induced by DDVP and the protective effects of CoQ10, CoQ10-NPS and NAC. Isolated rat hepatocytes (10^6 cells/mL) were incubated at 37°C in rotating round bottom flasks with 95% O_2 and 5% CO_2 in Krebs–Henseleit buffer (pH 7.4). *Different from control group ($P < .05$). ^aSignificantly different from DDVP-treated hepatocytes ($P < .05$). ^{a,b}Significantly different from DDVP and CoQ10 treated cells ($P < .05$). Data are shown as mean \pm SD for at least three different experiments. [Color figure can be viewed at wileyonlinelibrary.com]

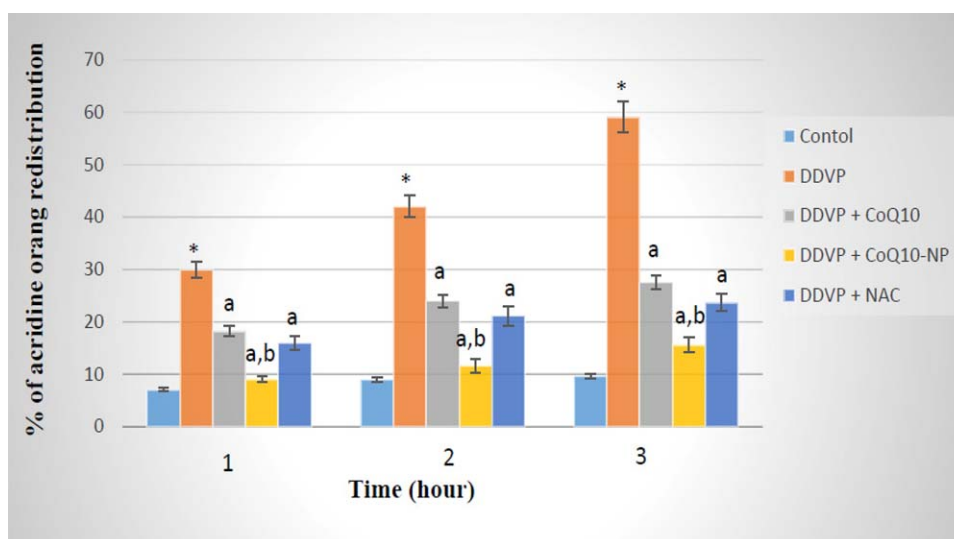


FIGURE 7 Lysosomal membrane damage induced by DDVP and the protective effects of CoQ10, CoQ10-NPS and NAC. Hepatocytes (10^6 cells/mL) were incubated in Krebs–Henseleit buffer pH 7.4 at 37°C for 3.0 h following the addition of DDVP (1 mM). Lysosomal membrane fragility was measured as fluorescent intensity unit of diffused cytosolic green fluorescence induced by acridine orange following the redistribution from lysosomes into cytosol in acridine-orange-loaded hepatocytes. *Different from control group ($P < .05$). ^aSignificantly different from DDVP-treated hepatocytes ($P < .05$). ^{a,b}Significantly different from DDVP and CoQ10 treated cells ($P < .05$). Data are shown as mean \pm SD for at least three different experiments. [Color figure can be viewed at wileyonlinelibrary.com]

To underline the hepatoprotective role of CoQ10 and its particulated form in liver damage, we utilized the accelerated cytotoxicity mechanism screening (ACMS) technique. In this method a high drug dose over 3 h exhibitions similar hepatotoxic effect *in vitro* as would do a lower drug dose over a longer period of time (24–48 h) *in vivo*,⁴⁰ which makes it useful to predict and characterize liver toxicity *in vivo*.

The superiority of CoQ10-NPs could be explained in this way that nanoparticle systems have a better aqueous solubility profile and a smaller size, so a higher concentration of the bioactive compound could be delivered into the cell compartment. Besides, due to the small size of CoQ10-NP, it is easily transported across hepatic sinusoid. While, elevated aqueous solubility renders the nanoparticle the superiority to provide a higher concentration in damaged liver tissue to better prohibition of the caspase signaling cascade to prevent hepatocyte cell death.

Mitochondria as cellular powerhouses are known to be the major energy sources and any damage to these organelles leads to the disruption of ATP producing cascades. Furthermore, constant leakage of electrons in the respiratory chain, turns mitochondria into a chief oxidative stress target in most mammalian cells.⁴¹ The present study demonstrated that the hepatocyte MMP was declined by DDVP which in turn led to the mitochondrial permeability transition pore (MPTP). Furthermore, delay in MMP collapse with antioxidants (NAC, CoQ10, CoQ10-NPs) proposed that MMP collapse was a consequence of ROS formation which is in accordance with published reports.^{42,43} Also, improved function of CoQ10-NPs in comparison with bare CoQ10 suggested the accumulation of sufficient concentration of CoQ10 juxta mitochondria. Moreover, enhanced penetration of CoQ-NPs into the mitochondrial membrane is another hypothesis which need further experiments to be proved completely.

TABLE 1 Effect of antioxidants on Intracellular GSH and GSSG during DDVP-induced hepatocyte damage

Addition	Intracellular GSH (nmol/ 10^6 cell)			Intracellular GSSG (nmol/ 10^6 cell)		
	60	120	180	60	120	180
Incubation Time	60	120	180	60	120	180
Control	57.4 \pm 3.2	52.2 \pm 2.8	49.1 \pm 4.2	5.1 \pm 2.5	6.9 \pm 5.2	10.1 \pm 6.4
DDVP(1mM)	40.7 \pm 5.4*	38.8 \pm 6.1*	34.7 \pm 3.4*	10.4 \pm 6.2*	14.9 \pm 5.4*	19.2 \pm 4.8*
DDVP (1mM) + CoQ10(300 μ M)	50.6 \pm 2.2 ^a	46.8 \pm 3.5 ^a	42.4 \pm 4.6 ^a	7.0 \pm 3.3 ^a	8.5 \pm 6.7 ^a	11.8 \pm 4.6 ^a
DDVP(1mM) + CoQ10-NPs(300 μ M)	56.2 \pm 4.3 ^{a,b}	51.9 \pm 2.3 ^{a,b}	49.2 \pm 5.9 ^{a,b}	5.2 \pm 5.2 ^{a,b}	6.9 \pm 4.4 ^{a,b}	9.8 \pm 3.9 ^{a,b}
DDVP(1mM) + NAC(200 μ M)	51.2 \pm 6.4 ^a	49.6 \pm 3.4 ^a	46.1 \pm 3.8 ^a	7.4 \pm 2.9 ^a	8.3 \pm 4.3 ^a	10.8 \pm 5.8 ^a

Hepatocytes (10^6 cells/mL) were incubated in Krebs–Henseleit buffer pH 7.4 at 37°C for 3.0 h following the addition of DDVP (1 mM). Intracellular GSH and GSSG was determined by HPLC analysis.⁴⁵ *Different from control group ($P < .05$). ^aSignificantly different from DDVP-treated hepatocytes ($P < .05$). ^{a,b}Significantly different from DDVP and CoQ10 treated cells ($P < .05$). Values shown are the means \pm SD of three separate experiments.

TABLE 2 Hepatoprotective effect of the CoQ10 and its nanoparticles on liver function markers in rats with DDVP-induced hepatotoxicity

Treatment	AST (U/L)	ALT (U/L)	GGT (U/L)
Control	54.1 ± 3.2	40.6 ± 4.8	2.7 ± 0.1
DDVP	96.5 ± 3.8 ^a	89.1 ± 5.1 ^a	10.4 ± 2.1 ^a
NAC + DDVP	83.4 ± 2.2 ^b	74.6 ± 3.3 ^b	6.7 ± 1.6 ^b
CoQ10 + DDVP	81.3 ± 4.2 ^b	72.3 ± 5.4 ^b	7.2 ± 2.3 ^b
CoQ10-NPs + DDVP	59.3 ± 5.6 ^{b,c}	54.5 ± 6.2 ^{b,c}	3.5 ± 0.8 ^{b,c}

DDVP: dichlorvos; NAC: n-acetylcysteine; CoQ10: Coenzyme Q10; CoQ10-NPs: Coenzyme Q10 nanoparticles; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma glutamyl transferase;

Values are mean ± SD for five animals in each observation.

^aSignificantly different from control group ($P < .05$).

^bSignificantly different from DDVP-intoxicated group ($P < .05$).

^cSignificantly different from DDVP groups treated with CoQ10 ($P < .05$).

DDVP administration has been shown to induce oxidative stress which is concomitant with decreased glutathione reductase, reduced and oxidized glutathione levels in different tissues.² In the current study, we observed a substantial GSH deprivation, which might be a mechanism for the DDVP-induced toxicity in isolated rat hepatocytes. Our results showed that antioxidants (NAC and CoQ10) are able to increase hepatocytes glutathione levels and this effect may also contribute to protection against DDVP-induced cytotoxicity. Also, for the

first time, this study demonstrated that potential antioxidant effect of nano-CoQ10 was considerably higher than CoQ10 in replenishment of GSH pools in DDVP-exposed isolated rat hepatocytes.

High amounts of intracellular lipophilic H_2O_2 are generated subsequent to the ROS formation in the mitochondria, which in turn diffuse easily in the cell and pass lysosomal membrane due to the inadequate detoxifying processes where in a Fenton type reaction with lysosomal Fe^{3+} , highly reactive hydroxyl radicals are produced leading to the LPO of membrane and lysosomal membrane leakiness. Defects in lysosomal membrane integrity result in the liberation of lysosomal digestive proteases into the cytosol. This toxic pathway triggers the oxidative cycle from mitochondria to lysosomes.

The protective effects of CoQ10 in lysosomal membrane integrity of isolated rat hepatocytes offer evidence that CoQ10 was found to contribute to both as a single-electron transporter and also a proton carrier in lysosomes similar to what happens in the respiratory chain of mitochondria. The reduction-oxidation of CoQ10 in lysosomal membrane boosts this organelle to enhanced cleaning of the cellular debris. Inhibition of DDVP-induced ROS production, MMP loss and lysosomal membrane damage by CoQ10, NAC and nano-CoQ10 shows the counter action of aforementioned agents on lysosomal, mitochondrial outer membrane permeability and all subsequent stages of the apoptotic cascade which is in line with previous investigations. These relevant outcomes recommend that there is a close-fitting link between mitochondria and lysosomes which possibly take part in DDVP-induced toxicity.

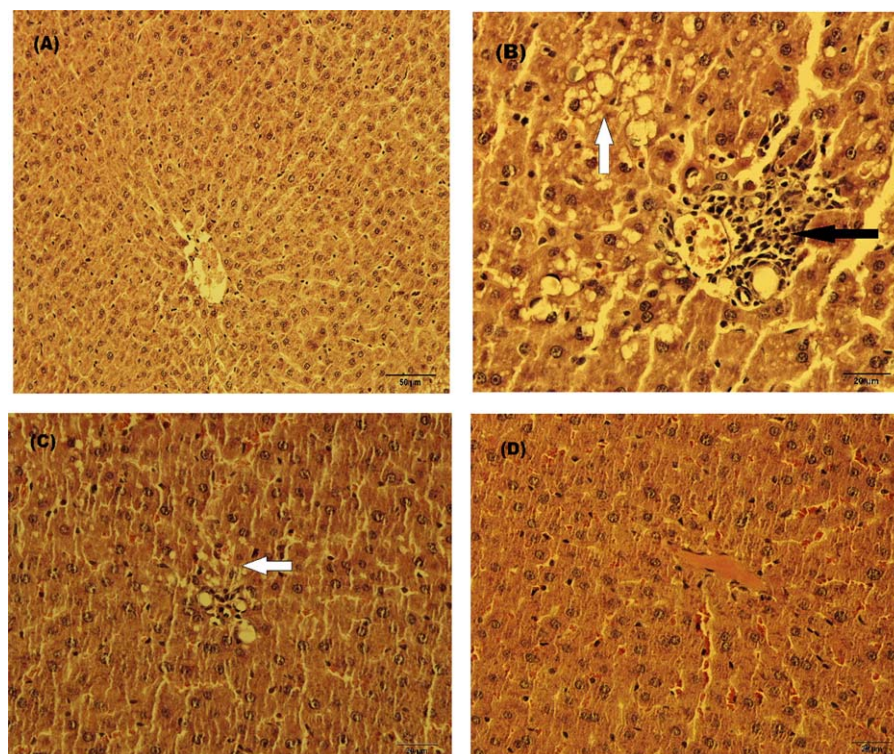
**FIGURE 8** Histopathological damage induced by DDVP (B) and the protective effects of CoQ10 (C) and CoQ10-NPS (D). DDVP administration resulted in hepatocyte degeneration, fatty change (white arrows) and inflammatory cell infiltration (Black arrows) in comparison with control group (A). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Effect of the NAC, CoQ10 and its nanoparticles on lipid peroxidation and antioxidant enzyme activities from the liver homogenate of rats with hepatotoxicity induced by DDVP

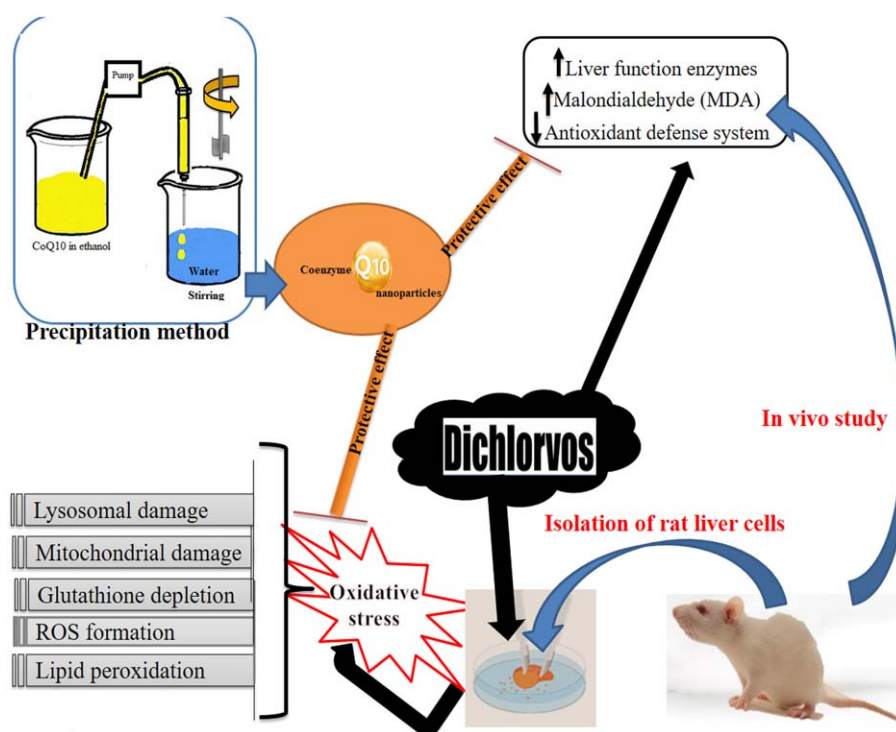
Treatment	SOD (U/mg protein)	CAT (K/mg protein)	GPx (U/mg protein)	MDA (nmol/mg protein)
Control	8.3 ± 0.9	75.2 ± 3.3	68.3 ± 3.1	113.4 ± 5.6
DDVP(1.6 mg/kg)	2.5 ± 0.3 ^a	29.5 ± 1.6 ^a	22.4 ± 1.9 ^a	221.6 ± 6.2 ^a
NAC (100 mg/kg) + DDVP	5.0 ± 0.4 ^b	45.9 ± 4.2 ^b	41.1 ± 3.6 ^b	156.8 ± 3.9 ^b
CoQ10 (50 mg/kg) + DDVP	4.1 ± 0.9 ^b	43.5 ± 3.6 ^b	37.5 ± 2.6 ^b	162.9 ± 6.2 ^b
CoQ10-NPs (50 mg/kg) + DDVP	6.6 ± 0.8 ^{b,c}	65.9 ± 2.5 ^{b,c}	56.1 ± 4.3 ^{b,c}	118.6 ± 4.2 ^{b,c}

DDVP: dichlorvos; NAC: n-acetylcysteine; CoQ10: Coenzyme Q10; CoQ10-NPs: Coenzyme Q10 nanoparticles; Values were expressed as mean ± SD for five animals in each observation.

^aSignificantly different from control group ($P < .05$).

^bSignificantly different from DDVP-intoxicated group ($P < .05$).

^cSignificant difference in DDVP groups treated with CoQ10 ($P < .05$).

**FIGURE 9** Schematic picture describing in an illustrative and comprehensive way the experimental set-up and the results/outcome. [Color figure can be viewed at wileyonlinelibrary.com]

Our *in vivo* studies showed that DDVP induced hepatotoxicity in rats, which were associated by the increment of liver function enzymes and decrease in the activity of antioxidant enzymes.

Regarding our *in vivo* work, DDVP has a significant effect in mitigation of liver antioxidant enzyme levels.

H₂O₂ is produced via the function of SOD followed by induction of hydroxyl radicals throughout Fenton and/or Haber-Weiss reactions if the agent is not removed by CAT and/or GSH.⁴⁴ Exposure to organophosphate pesticides is often followed by impairment of the antioxidant defense and characterized by the depletion of GSH. In the current study, we showed that NAC, CoQ10 and its nanoparticles counterpart played detoxifying roles in metabolizing the xenobiotics through enhancement of SOD, CAT, and GPx levels to scavenge the ROS

radicals and MDA levels generated from DDVP. Furthermore, our data also proposed that pretreatment with NAC, CoQ10 and CoQ10-NPs effectively reduced the leakage of AST, ALT and GGT and prevented the DDVP-intoxication-mediated progression of hepatotoxicity which were also apparent in the histopathological results. The effects of CoQ10-NPs in DDVP-induced liver toxicity were summarized in Figure 9.

5 | CONCLUSION

The outcomes of this study revealed the hepatoprotective role of CoQ10-NPs as well as its enhanced antioxidant effect to improve

CoQ10 medical application due to its poor absorption and bioavailability in oral administration. Many researchers have attempted to increase the effect CoQ10 on mitochondrial transport chain to combat drug and/or toxin-induced mitopathy and to reduce ROS production in recent years. In this regard, mitigation of hepatotoxicity caused by DDVP in which oxidative stress and mitochondrial malfunction are shown to be the major mechanisms of toxicity was studied and treatment of hepatocytes with CoQ10-NPs, CoQ10 and NAC as putative antioxidants inhibited DDVP-induced toxicity while the nano-formulated form of CoQ10 presented a more potent impact in neutralization of DDVP-associated side effects in rat hepatocytes. Hence, the results of this investigation suggest a new strategy to protect against organophosphate pesticide toxicity in the liver and maybe other organs. In summary, CoQ-NPs are deemed to be a safe supplementary therapeutics in pathologies related to xenobiotics-induced liver injuries.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest associated with this work.

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